

## SPECTROSCOPIC FLUID ANALYZER

### FIELD OF THE INVENTION

[0001] The present invention relates to the field of the qualitative analysis of milk, using spectroscopic techniques in the visible and near infra-red, especially for on-line assessment of the component parts of the milk during the milking process.

### BACKGROUND OF THE INVENTION

[0002] Measurement of the component parts of milk, and real time knowledge of the results of these measurements is an important factor in the efficient management of a dairy farm. Knowledge of the levels of almost all of the various component parts of the milk is important for different aspects of the herd management. These components include fat, total protein, casein, lactose, somatic cells, blood, progesterone, amino-acids urea, and nucleic acid. The fat and protein content, for instance, are major factors in determining the price that the farmer will obtain for his milk, because they are important economic indicators of the overall milk quality. Changes in these values can indicate to the farmer an incorrect diet. Thus, changes in the fat content could indicate an imbalance in the forage to concentrate ratio in the feed. Low total protein level may indicate a dietetic energy deficiency. The somatic cell count and the blood count on the other hand, may be used as diagnostic indicators of a specific clinical state of the cow. Lactose content is generally very stable for a given cow, unless mastitis is present.

Several methods are described in the prior art for performing on-line milk analysis, with the object of more efficient milk production and herd management. The use of near infrared (NIR) spectroscopy for analyzing milk has been known for almost 15 years, and the early methods used laboratory-type NIR spectrometers for

analyzing the milk off-line. A number of such instruments are available commercially, but they are expensive, and their use is thus generally limited to centralized laboratories, to which the farmer would send milk samples for testing typically only once a month.

[0003] In the article "Near Infra-Red Spectroscopy for Dairy Management: Measurement of Unhomogenized Milk Composition" by R. Tsenkova et al., published in Journal of Dairy Science, Vol. 82, pp. 2344-2351, 1999, there is proposed a method whereby the milk content is spectroscopically analyzed in the NIR range of from 400nm to 2500nm. A proposal is made therein to use fiberoptic probes and relatively inexpensive silicon detectors for detecting the radiation within the range 700nm to 1100nm, thereby making the method affordable enough to be applied at the milking station for real-time analysis during milking. However, no details are given of an apparatus suitable for performing this analysis using such silicon detectors. Furthermore, although the use of inexpensive detectors is proposed, no mention is made of the sources that could be used with these detectors, for providing the NIR illumination.

[0004] The article presents an analysis and comparison of the results obtained in the 1100 to 2400nm spectral range, to those obtained in the 700 to 1100nm spectral range, where inexpensive silicon detectors can be used. Essentially continuous measurements (every 2 nm) were made across the whole of these spectral ranges. Though not specifically stated in the article, such spectral coverage can generally be obtained from the internal blackbody illuminating source of most NIR spectrometers. The methods described in the Tsenkova et al. article are largely directed at statistical methods of extracting the desired concentration levels from the overall absorption spectra. A commercial software program was used to develop models for determining fat, total protein and lactose content, and calibration of the models was performed using the Partial Least Squares (PLS) regression technique.

[0005] Further descriptions of methods of milk analysis using NIR spectroscopy are given in the articles "Fresh raw milk composition analysis by NIR spectroscopy" by Z. Schmilovitch et al, published in Proceedings of the International Symposium on the Prospects for Automatic Milking, Wageningen, Netherlands, EAAP Publication No. 65, pp. 193-198 (1992), and in "Low Cost Near Infra-red Sensor for On-line Milk Composition Measurement", by Z. Schmilovitch et al., published in the Proceedings of the XIV Memorial CIGR World Congress, 2000, Tsukuba, Japan, some of the authors of which are co-applicants for the present invention.

[0006] The spectroscopic measurements themselves in the above-mentioned Tsenkova et al article were performed off-line, on collected samples, using a commercial NIR spectroscopic milk analyzer, the Milko Scan, supplied by Foss-Electric A/S, Hillerod, Denmark. The cost of such instruments is such that they are only generally feasibly economical for installation in central laboratories, and not in every cowshed, let alone at every milking station.

[0007] There therefore exists an important need for an inexpensive and simple apparatus and method for the on-line qualitative analysis of milk, which will be sufficiently inexpensive that it can be widely used to enable real-time data to be obtained during the milking process, even at each milking station, but without significantly compromising the accuracy of the measurements required for efficient dairy herd management. Furthermore, the apparatus must be capable of performing its analyses on the type of milk flows typically obtained from milking machines. Such flows are highly pulsed in nature, and generally very turbulent, such that a conventional optical sensing path, which measures the optical transmission through the flow from side to side of the flow channel, is of limited use.

[0008] The disclosures of all publications mentioned in this section and in the other sections of the specification, and the disclosures of all documents cited in the above publications, are hereby incorporated by reference, each in its entirety.

## SUMMARY OF THE INVENTION

[0009] The present invention seeks to provide a novel fluid analyzing system, which uses a near infra-red spectroscopy technique for determining the percentage concentrations of the constituent parts of the fluid. The analyzing system is particularly useful for the analysis of milk. The NIR measurement technique, according to various preferred embodiments of the present invention, differs from techniques previously used for milk qualitative analysis in a number of ways.

[0010] According to a first preferred embodiment of the present invention, the source of incident illumination for the spectroscopic absorption measurements is made up of a series of light emitting diodes (LED's), each having its own preselected center wavelength. These wavelengths are preferably selected such that the LED's have overlapping spectral widths, such that the entire spectrum over which measurements are to be taken is covered. Since the typical spectral width of a LED in these spectral regions is of the order of up to 40 to 60nm half-width, according to one preferred embodiment of the present invention, a series of 10 LED's covers the desired spectral measurement range of the visible and NIR spectral ranges from 450 - 950nm. The LED's illuminate the fluid sample sequentially, and the transmission absorbance through the sample is measured by means of photodetectors. According to another preferred embodiment of the present invention, in addition to the transmission absorption measurements, the reflectance or scattering from the sample is measured for the wavelength range of each LED.

[0011] LED's, like the photodiodes described in the Tsenkova et al. article, are inexpensive light sources, readily available and conveniently applicable. The use of a series of LED's as light sources, according to this preferred embodiment of

the present invention, thus complements the previously proposed use of inexpensive photodetectors, in enabling the construction of an inexpensive and convenient-to-use NIR absorption spectrometer for on-line milk analysis.

[0012] There is also provided, according to yet another preferred embodiment of the present invention, a method of illuminating the sample chamber to perform NIR spectroscopy on a fluid mixture such as milk, using a plurality of LED's, which cover the visible and NIR spectral ranges. The LED's illuminate the fluid sample sequentially, and the transmission absorbance is measured for each incident LED. Additionally and preferably, the reflected radiation is detected at an angle to the incident radiation. According to one preferred embodiment, it is measured at right angles to the incident radiation. According to a second preferable embodiment, back-reflected radiation is detected, by positioning the detector essentially at the same location as the input light. This enables a convenient arrangement of source and detector to be used.

[0012] In conventional absorption spectroscopy, the absorptions are related to the concentrations of the component parts of the fluid by means of the familiar Beer-Lambert law, which assumes an exponential relationship between the light intensity absorption and concentration. According to the preferred methods of the present invention, these concentrations of the component parts of the fluid are expressed in the form of an expression, which is more preferably a polynomial expression, the expression being a function of the measured transmitted and/or reflected or absorbed intensities, and of empirical coefficients, which are extracted preferably by performing a Partial Least Squares or a Ridge Least Squares regression technique on measured intensities, obtained by measuring a large number of test samples having known concentrations of the component, as is well known in the art. However, according to further preferred embodiments of the present invention, the regression method used, differs in two respects from the previously used techniques, in that:

- (i) it is performed on a polynomial of up to third order in intensities, where the second order intensity terms arise from the second harmonic measurements and the third order intensity terms arise from the third order harmonic measurements; and
- (ii) the polynomial includes terms arising both from transmission absorbance spectroscopy measurements and from reflection spectroscopy.

[0013] Though in this preferred embodiment of the present invention, PLS or RLS regression techniques are proposed in order to extract the coefficients of the third order polynomial, it is to be understood that this technique is only one of several statistical techniques available for fitting such a polynomial to the absorbance data, and it is not intended that this invention be limited to the use of the PLS or the RLS technique for the statistical analysis. Furthermore, although a polynomial of third order in intensity is used in the embodiment described, it is to be understood that any higher order polynomial can be used, and that it is not intended that this invention be limited to the use of a third order polynomial in intensity.

[0014] The empirical coefficients thuswise obtained for each sample are stored as a database in the computing system memory, along with the component concentrations of the associated sample. When an unknown sample of milk is to be measured, during use of the system, the extraction of the concentrations of the constituents of the sample is preferably performed by a further statistical analysis method using the contents of this database, such as is known from chemometric analysis methods used in the analysis of multiple component chemical reaction dynamics.

[0015] The spectroscopic measurements are performed on the milk using a novel sampling chamber, constructed and operative according to a further preferred embodiment of the present invention. This sampling chamber has a recessed cavity, preferably adjoining the main flow line of the milk, and located in a generally

downwards direction, such that it fills with a constantly changing sample of the flowing milk. This enables optical transmission measurements to be performed on a pulsating milk flow, without the pulsation and turbulence significantly affecting the accuracy of the measurement. The optical beam measurement path traverses the central area of this recessed cavity, with the source on one side and the transmission measurement detector on the other side. For the purpose of the scattering measurements, another detector is disposed, preferably at right angles to the optical beam path, to detect light scattered at 90 degrees by the milk in the sampling chamber. For the purpose of the back-scattering measurements, another detector is disposed, preferably at approximately the same location as the source LED's.

[0016] In accordance with yet another preferred embodiment of the present invention, there is also provided a system for determining the concentration of at least one component of a fluid, the fluid comprising at least two components having different optical absorption properties, the system comprising a sample chamber containing the fluid, a plurality of illumination sources, at least one of which, when excited, emits light in an essentially continuum of wavelengths, at least two of the sources having different spectral ranges of emission, the sources being disposed such that the light from the sources is incident on the fluid in the sample chamber, a first detector disposed such that it measures the intensity of the light transmitted through the fluid, a second detector disposed such that it measures the intensity of the light scattered from the fluid, a control system which excites at least two of the illumination sources serially, such that the fluid is separately scanned with wavelengths of the light of the at least two illumination sources, and a computing system operative to determine the concentration of the at least one component of the fluid from the intensity of the light transmitted through the fluid and the light scattered from the fluid. The sources may preferably be light emitting

diodes. The second detector may preferably be disposed such that it measures the intensity of light reflected from the fluid.

[0017] According to another preferred embodiment, in the above-mentioned system, the computing system is operative to determine the concentration by fitting the intensity of the light transmitted through the fluid and of the light scattered from the fluid to a polynomial expression for the concentration of one of the components in terms of the intensities. The polynomial expression is preferably of at least second order in the transmitted and scattered intensities. Furthermore, the transmitted and scattered intensities may preferably be related to the concentration of the component by means of empirical coefficients determined by a statistical analysis of transmitted and scattered intensities obtained from a plurality of samples of the fluid having known concentrations of the component. This statistical analysis may preferably be a Partial Least Squares regression method or a Ridge Least Squares regression method. In addition, the empirical coefficients are preferably stored in a database, and the concentration is extracted from the transmitted and scattered intensities by means of statistical analysis methods operating on the database.

[0018] Any of the above-mentioned systems may preferably be utilized for analyzing milk, and the system may preferably determine the constitution of the milk on-line during the milking process.

[0019] According to even another preferred embodiment of the present invention, there is also provided a method of determining the concentrations of at least one component of a fluid, the fluid comprising at least two components having different optical absorption properties, comprising the steps of :

- (a) illuminating the fluid with incident light from a source essentially having a continuum of wavelengths of emission,
- (b) measuring on the fluid transmitted and scattered intensities of the incident light,
- and

(c) fitting the intensities to a polynomial expression for the concentration of the component in terms of the intensities, the polynomial expression being at least second order in the transmitted and scattered intensities.

[0020] According to yet another preferred embodiment of the present invention, in the above-mentioned method, steps (a) and (b) may be repeated using a plurality of sources, each source having its own continuum of wavelengths. The polynomial expression may preferably be of third order in the transmitted and scattered intensities. Furthermore, the scattered intensities may be reflected intensities. In addition, the transmitted and scattered intensities may preferably be related to the concentration of the component by means of empirical coefficients, which are determined by a statistical analysis of transmitted and scattered intensities obtained from a plurality of samples of the fluid having known concentrations of the component. This statistical analysis may preferably be either a Partial Least Squares regression method, or a Ridge Least Squares regression method.

[0021] In the methods described above, the empirical coefficients may preferably be stored in a database, and the concentration extracted from the transmitted and scattered intensities by means of statistical analysis methods operating on the database.

[0022] In any of the above-described methods the fluid may preferably be milk.

[0023] There is also provided, according to yet another preferred embodiment of the present invention, a sampling chamber for performing optical measurements on a sample of a flowing fluid comprising, a flow line for the passage of the fluid, a recessed cavity in fluid contact with the flow line, and directed in a generally downward direction such that a sample of fluid in the flow line can enter the cavity, and an optical transmission path passing through the cavity in such a position that it lies outside the confines of the flow line.

[0024] In the above-described sampling chamber, the optical transmission path preferably comprises an entry port for inputting a light beam from a source, and an exit port for outputting light to a detector. The input port and the output port are preferably disposed such that the light beam traverses the sampling chamber linearly, such that the sampling chamber can perform transmission optical measurements. Alternatively and preferably, another output port may be disposed at an angle to the direction of the light beam, such that the sampling chamber can perform scattering optical measurements. Furthermore, the exit port may be disposed essentially co-positional with the input port such that the sampling chamber can perform back-scattering optical measurements.

[0025] According to yet further embodiments of the present invention, the recessed cavity is such that the sample is repeatedly changed by the effects of the flow of the fluid in the flow line. It may also be such that the optical measurements are generally unaffected by turbulence or pulsations in the flow line.

[0026] In the above-mentioned sampling chamber, the optical measurements are preferably utilized to determine relative concentrations of components of the fluid.

[0027] Furthermore, the fluid flowing through the above mentioned sampling chamber may preferably be milk.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The present invention will be understood and appreciated more fully from the following detailed description, taken in conjunction with the drawings in which:

[0029] Fig.1 is a schematic diagram of the optical measurement system of a spectroscopic milk analyzer, constructed and operative according to a preferred embodiment of the present invention;

[0030] Fig. 2 is an enlarged view of the illumination rosette in the measurement system shown in Fig. 1;

[0031] Figs. 3A and 3B are schematic drawings of a sampling chamber, according to another preferred embodiment of the present invention, for performing optical measurements on a sample of a flowing fluid. Fig. 3A is a side view of the sampling chamber, while Fig. 3B is a cross-section;

[0032] Fig. 4 is a graph showing the results of approximately 140 analyses of samples of milk, taken from different cows during milking, for the protein concentration, using a milk analyzer constructed and operative according to preferred methods of the present invention; and

[0033] Fig. 5 is a graph showing the results of the analysis of approximately 140 samples of milk for the somatic cell count (SCC), using a milk analyzer constructed and operative according to a preferred embodiment of the present invention.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0034] Reference is now made to Fig. 1, which is a schematic illustration of a spectroscopic milk analyzer and its optical measurement system, according to one preferred embodiment of the present invention. The milk flows through a flow tube 10, typically on its way from the milking station 12 towards the collection point 14, though it is to be understood that the milk analyzer of the present invention could be advantageously incorporated at any other point in the milk flow path. A sampling chamber 16 is located in the milk flow line, and a sample of the flowing milk collects in the chamber 18. The structure and operation of the sampling chamber are described in more detail hereinbelow, in connection with Figs. 3A and 3B.

[0035] Near the sampling chamber is provided a Light Emitting Diode (LED) array 20 which preferably incorporates a number of discrete LED emitters 21, each emitting at a different wavelength within the range to be used for the measurement. According to one preferred embodiment, the wavelengths of the LED's used range from 450nm to 950nm, to cover the visible to NIR regions of the spectrum. According to this preferred embodiment, the light output from each LED is transmitted by means of an optical fiber 22 to a rosette 23, where all the fibers are bundled together to form a compact source which emits the wavelength of whichever LED or LED's are illuminated. In the center of the rosette there is located a detector 24.

[0036] Reference is now made to Fig. 2, which is a schematic view of the illumination rosette 23 of Fig. 1, enlarged to show the details more clearly. The ends 25 of the fibers 22 which emit the LED illumination, are grouped as closely as possible to each other, so that the different wavelength sources are as close as possible to a single source. At the center of this bundle is positioned a detector 24, for use in detecting back-scattered light. In operation, each of the LED's is turned on sequentially, such that the spectrum of discrete wavelength points is measured sequentially. According to another preferred embodiment of the present invention, another such detector 29 is positioned at an angle to the direction of the incident light, so that scattered light in directions other than that of back-scattered reflection can preferably be detected.

[0037] The light transmitted from the source rosette 23 through the sample chamber is detected, preferably by means of a silicon photo-detector 27 disposed opposite the light output port 31. The detected signal 28 is input into a signal amplification and processing system 30. This can optionally be operated as a phase sensitive detection system, in order to provide optimum detection sensitivity, with the LED's modulated accordingly. The output intensities from the detection system are preferably fed to a computing and control system 32, where the spectra

obtained are analyzed by methods according to more preferred embodiments of the present invention. The computing and control system 32, also preferably passes control information 35 to the LED sources 21, both to provide the modulation frequency, if used, which is also input by means of control line 36 to the phase sensitive detector in the signal amplifying and processing system 30, and to control the switching order and timing of the LED sources 21, for scanning the complete spectral range to be measured.

[0038] According to one preferred scanning program, each LED is turned on for several milliseconds, and the absorption and/or scattering measurements are performed at that wavelength. In order to perform the measurements more rapidly, the transmission absorbance signal on detector 27 and the back-reflection signal on detector 24 are measured simultaneously. If a right-angled detector 29 is used, its signal is also preferably measured simultaneously with the signals on detectors 24 and 27. A complete scan of all 10 of the preferably used LED's takes approximately 250 msec with the research apparatus used to develop and measure the performance of the invention. Use of a high speed microprocessor running dedicated software for processing the signals, as would preferably be incorporated into a commercial milk analyzer constructed according to the present invention, is expected to reduce the scan time.

[0039] The pulse rate of the milk flow through the chamber during milking is at most generally no faster than one milk pulse every two seconds. Since this repetition rate is generally significantly slower than the measurement scan rate, the absorbance/reflectance measurements can preferably be repeated several times on each milk sample collected in the sample chamber, and then averaged for each sample, thereby reducing the noise level of the measurements, and increasing the accuracy with which the concentrations can be calculated.

[0040] According to a further preferred embodiment of the light generation and detection system of the present invention, the fiber optic cables may be

replaced with plastic light guides to form a less costly, more compact, and waterproof assembly. The light guides preferably have a siphon-like structure, having a 5 mm diameter at the LED end, tapering to 1mm at the sample chamber.

[0041] In order to overcome physical size constraints, the 10 LED's are preferably divided into two groups of five, each rosette having only 5 LED's and its own central detector, and each rosette being disposed on opposite sides of the sample chamber. According to this preferred embodiment, illumination and detection is performed sequentially from both sides of the sampling chamber, with the 5 LED's in each rosette. The detector of each rosette collects back scattered light from it's own LED's and transmitted light from the LED's of the opposite rosette. According to this preferred embodiment, the function of detector 29, if used, remains unchanged.

[0042] A major problem in analyzing the spectrum of a multi-component fluid such as milk, arises from the overlap of the individual absorption and scattering spectra of each of the separate components. Furthermore, according to the illumination method of the present invention, where the spectral width of the LED sources may be such as to include a number of such individual absorption and scattering spectral lines, in order to quantitatively analyze the milk for its separate constituents, a method must be provided for extracting this information about the identification of the lines present, and determination of their intensity. The method must be capable of doing this to a plurality of lines "hidden" within the intensity measurements obtained from the relatively broad bandwidth LED sources.

[0043] According to a preferred method of the present invention, a high order polynomial expression is used to express the concentrations,  $C$ , of the various milk constituents in the sample chamber, in terms of the measured transmitted and reflected (scattered) light intensities for each LED measurement, each intensity term appearing with an empirical coefficient. According to one preferred embodiment, the polynomial may be of the form:

$$C\% = \sum a_{tj} I_{tj} + \sum b_{tj} I_{tj}^2 + \sum c_{tj} I_{tj}^3 + \dots + \sum a_{rj} I_{rj} + \sum b_{rj} I_{rj}^2 + \sum c_{rj} I_{rj}^3 + \dots$$

where:

$j = 1-10$ , representing 10 discrete light sources in the NIR and visible spectrum;

$I_{tj}$  = intensity of the light from source  $j$ , detected on the transmittance photo-detector;

$I_{rj}$  = intensity of the light from source  $j$ , detected on the reflectance photo-detector;

$C\%$  = concentration of constituent  $C$ ; and

$a_{tj}$ ,  $b_{tj}$ ,  $c_{tj}$ .....,  $a_{rj}$ ,  $b_{rj}$ ,  $c_{rj}$ ....., = empirical coefficients, relating the intensities of the light detected to the concentration of the constituent  $C$ . According to one particularly preferred embodiment, a third order polynomial is used, and only coefficients up to  $c_{tj}$  and  $c_{rj}$  are used.

[0044] The values of these empirical coefficients are initially experimentally determined preferably by using a statistical analysis method, such as by performing PLS regression or RLS (ridge least square) calculations on a large database of absorption and reflectance data acquired experimentally from a large number of samples of milk with different and variable constituents. To provide a sufficiently broad database, the samples are preferably obtained from several hundred different cows. The data are obtained from absorption and reflectance measurements made using the light emitted from the ten LED's. The constituents of each sample of milk are independently determined, preferably using a standard spectrophotometric method, and these known constituent concentrations are then used to extract the empirical coefficients, by using a preferred statistical analysis method.

[0045] Once these coefficients are known, they are stored, along with the concentrations of the sample with which they are associated, as a reference

database in the computing system memory for use in measurements of unknown samples. The extraction of the concentrations of the constituents from an unknown sample of milk is preferably performed by a further statistical analysis method, comparing the measured intensities with the contents of the database, such as is known from chemometric analysis methods used in the analysis of multiple component chemical reaction dynamics. According to one preferred embodiment of the present invention, the analyzer uses ten LED sources, such that 20 measurement signals are obtained from each unknown sample of milk, 10 from transmission measurements, one from each of the 10 LED's, and 10 from reflectance measurements, one from each of the 10 LED's. These 20 measurement signals, each at their known wavelength range, are then related, preferably by means of the statistical analysis chemometric-type methods, to the large database of stored spectral curves related to various milk compositions, and from the analysis, a unique set of concentrations of the constituents of the milk sample is determined. This method of calibration and analysis thus allows the use of inexpensive LED's with their non-uniform wide spectral range, as light sources, rather than a more discrete and monochromatic source of light, such as a laser, as is used in some prior art optical fluid analyzers.

[0046] The results of these concentration analyses for all of the milk components detected, are preferably printed or displayed on the output unit 34 and transferred to the herd management system for analysis.

[0047] Reference is now made to Figs. 3A and 3B, which are detailed schematic drawings of the sampling chamber 16 for performing optical measurements on a sample of a flowing fluid, as shown in Fig. 1, constructed and operative according to another preferred embodiment of the present invention. Fig. 3A is a side view of the sampling chamber. For the preferred embodiment of a milk sampler, the milk enters the sampling chamber, preferably directly from the milking machine, by means of an input tube, 40. It then flows along the main flow

line 42. Adjoining the main flow line 42 is a recessed sample cavity 44, having a smooth profile 46 preferably in the shape of an arc of a circle. The cavity enters the main flow line along one side wall 48 of the flow line. The milk flows out of the sampling chamber by means of an output tube 50. In use, the sampling chamber is constrained in orientation such that the sampling cavity 44 is located in a generally downwards direction. As a result, the cavity fills with a sample of the flowing milk. In particular, each new pulse of milk entering the chamber sweeps out the previous sample, replacing it with a new sample, such that the sample is continually changed, and each pulse is analyzed, regardless of the regularity with which the pulses arrive. The sampling process is thus virtually continuous. This property of the sampling chamber is particularly important for a milk farmer since the milk components can change during the course of a single milking of a cow, and only such a continuous sampler can easily track these changes in real time. This enables the milk farmer, for instance, to divert the milk flow away from the main collection vat, if any degradation in the milk from its required standard is detected.

[0048] Near the center of the sampling cavity, and below the line of the bottom wall of the flow line, there is situated a bore 52 in the body of the milk sampler, through which light can pass on its way to the sampling cavity 44. In the preferred embodiment shown, the bore 52 is filled with a transparent solid, through which the illumination passes, thereby preventing the inflow of milk to the bore. Alternatively and preferably, light guides located where the bores meet the sampling chamber may be used for this purpose. On one side of this bore there is a light source mount 54, in which or to which is attached the LED illumination rosette 23 providing the incident illuminating beam. The optical beam measurement path traverses the central area 56 of the recessed cavity, and at the end of the bore remote from the source, there is located a detector mounting 58, in which is installed the transmission measurement detector 27. According to a further preferred embodiment of the present invention, in addition to the transmission

measurements, back-scattering measurements may be performed by the use of a detector located as close to the source as possible, ideally at essentially the same position as the source. This can preferably be achieved by mounting the detector at the center of the rosette 23, attached to the sample chamber by a mounting assembly 62. According to yet another preferred embodiment of the present invention, the optical measurement may be made by scattering other than back-scattering, and for this purpose, another detector mount 60 is disposed, preferably at right angles to the optical beam path, to detect light scattered at 90 degrees by the milk in the sampling chamber. According to yet another preferred embodiment of the present invention, the transmission detector 58 is replaced by another rosette 23 including both multiple light sources and a detector, as described above.

[0049] By means of the milk sampler shown in Figs. 3A and 3B, it is possible to perform optical transmission measurements on a pulsating milk flow, without the pulsation and turbulence significantly affecting the accuracy of the measurement. Furthermore, the measurements can be made on-line and in real time, thus providing the farmer or milking data collection system with instant information about any change in the milk composition.

[0050] According to a further preferred embodiment, the sampling chamber can be made the only part of the system installed in the potentially problematic environment of the milking stall (dirt, manure, water), and the LED sources and the detectors, and the electronic units, installed remotely, and connected by means of optical fibers or light guides and cables to the sampling chamber. Furthermore, since the optical and electronic system does not need to perform analyses constantly during each milking, and also not, for instance, when the occupant of each stall is being changed, it is possible to multiplex several sample chambers in different milking stalls to one central optical and control system. As a result, even though the analyzer of the present invention is of low constructional cost compared with prior art analyzers, it becomes possible to even further reduce the cost to the

farmer of a system which provides on-line milk analysis on the complete herd at the time of milking.

[0051] The width 64 of the sampling cavity, which is defined as the optical path length through which the light passes within the cavity, is an important parameter in determining the accuracy and sensitivity of the optical measurements on the sample. This is particularly relevant for a fluid such as milk, which, because of its comparative opacity, arising from the emulsive nature of milk, attenuates any incident light in very short distances. This attenuation effect is also very dependent on the wavelength range used. The accuracy of the absorption measurement is inversely proportional to the distance light has to travel through the sample. For milk, for example, using direct absorption spectroscopy in the NIR-visible region, it is very difficult to accurately measure protein content for optical path lengths of more than approximately 2 mm. Thus, for instance, in the Tsenkova et al. article, it was noted that in the 700 to 1100 nm spectral region, though the best accuracy for fat determination was obtained with optical path lengths of 10 mm, for the determination of protein content such a long optical path length resulted in an accuracy which was inadequate for practical application. An optical path length of 1mm was found to be optimum for protein determination at those wavelengths. Though Tsenkova et al. found that the results in the 1100 to 2400 nm range were generally better than in the 700 to 1100 nm region, the latter region is to be preferred for use in a low cost instrument, because of the wide availability of low cost sources and detectors. The analysis of milk at wavelengths below about 900nm is particularly problematic because the flat and featureless nature of the milk spectrum in this region makes it difficult to obtain information from absorption measurements, such as are used in prior art methods.

[0052] Such conflicting measurement condition requirements may make it difficult to determine the optimum overall conditions for operation of a prior art spectrometric milk analyzer measuring only at the incident wavelength. In order to

overcome the problem of the high optical absorption of milk, some prior art spectroscopic milk analysis methods use back scattering measurements only. This approach too is problematic in that the real distance of penetration of the illumination is not really known, and the analysis results may therefore be inaccurate.

[0053] These problems are substantially reduced by the use of the preferred methods according to the present invention, where measurements of both transmission absorption, and of back reflection substantially reduce the dependence on the sampling cavity width. Thus, in association with known electronic noise filtering techniques, it has been found that optical path lengths of 5 or 10 mm may be used, and provide good accuracy for all of the components of milk. These methods have been found to be especially advantageous for protein measurements, which, in order to provide accurate results, have generally been performed using narrow chambers of up to 2mm with the prior art absorption methods.

[0054] Reference is now made to Fig. 4, which is a graph showing the results of approximately 140 analyses of samples of milk, taken from different cows during milking, for the protein content, using a milk analyzer constructed and operative according to preferred methods of the present invention. In Fig. 4, the protein concentration of the milk samples, as accurately measured on a standards laboratory type of analytical instrument, are shown as small open circles. The protein concentrations measured on the milk analyzer, according to the present invention, are shown by the × symbols. As is observed, the results of the analyses obtained in real time using the analyzer of the present invention, are very close to those obtained on the laboratory analytical instrument. The standard deviation of the measurements is approximately 0.04%.

[0055] Reference is now made to Fig. 5, which is a graph showing the results of approximately 140 analyses of samples of milk for the somatic cell count (SCC), shown in units of thousands of cells detected, using a 10-LED milk analyzer

constructed and operative according to preferred methods of the present invention. The SCC measurement is comparatively more difficult to perform accurately than the protein content analysis shown in Fig. 4, and yet the results shown in Fig. 5 indicate that the apparatus of the present invention, is capable of providing even this measurement with good accuracy. The standard deviation of the SCC measurements is approximately 23.6.

[0056] Similar sets of measurements on approximately 150 samples of milk to analyze lactose, fat and urea content show similar levels of accuracy, with the values of the standard square deviation,  $R^2$ , being  $R^2 = 0.970$ ,  $R^2 = 0.992$  and  $R^2 = 0.991$  respectively for these three sets of measurements.

[0057] It is thus observed from the above-mentioned results that the apparatus of the present invention, using inexpensive light sources and detectors, is capable of providing analyses on a flowing sample of milk, at the time of milking, with an accuracy which does not fall far from that of laboratory types of milk analysis instruments.

[0058] It will be appreciated by persons skilled in the art that the present invention is not limited by what has been particularly shown and described hereinabove. Rather the scope of the present invention includes both combinations and subcombinations of various features described hereinabove as well as variations and modifications thereto which would occur to a person of skill in the art upon reading the above description and which are not in the prior art.